IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Peter M. Glazer and Pamela A. Havre

Serial No: Continuation of 08/083,088

Express Mail Label
No. EL 709 418 853 US

Filed: February 14, 2001

Date of Deposit: February 14, 2001

For: CHEMICALLY MODIFIED OLIGONUCLEOTIDE FOR SITE-DIRECTED MUTAGENESIS

BOX PATENT APPLICATION
Assistant Commissioner of Patents
Washington, D.C. 20231

REQUEST FOR APPROVAL OF DRAWING CHANGES AND PRELIMINARY AMENDMENT

Sir:

I. Request for Approval of Drawing Changes

Pursuant to 37 C.F.R. § 1.121(a)(3), applicants respectfully request approval for changes to the drawings indicated in red on the attached photocopies of the informal drawings of Figures 1-9, and respectfully request entry of the following amendment to conform the specification to the requested changes to the drawings.

Applicants no longer have the original photographs for Figures 2, 5b, 6, and 8 in parent application U.S.S.N. 08,083,088 filed June 25, 1993. Therefore, the changes are made to cancel Figures 2, 5b, 6, and 8, and to relabel the drawings accordingly. Figures 3a and 3b are now Figures 2a and 2b. Figure 4 is now Figure 3. Figure 5a is now Figure 4. Figure 7, which is

described in the specification as Figures 7A and 7B, is now Figures 5A and 5B, to conform to the description in the specification, as amended. Figure 9 is now Figure 6.

Accordingly, an amendment to the application is required to refer to the renumbered figures. Pursuant to 37 C.F.R. § 1.84(u), applicants have deleted references to the canceled figures in the specification to correspond to the new labeling.

II. Preliminary Amendment

Prior to examination, please amend the application as follows.

In the Specification

On page 1, after the title and before "Background of the Invention", please insert the following paragraph:

--This application is a continuation of U.S. Serial No. 08/083,088 filed June 25, 1993.--

On page 5, line 17, after "pso-AG10 (4'" and before "hydroxymethyl", please insert a hyphen ("-").

On page 5, line 18, after "trimethylpsoralen-^{5} AGGAAGGGGG^{3})", please insert --(SEQ ID NO:1)--.

On page 5, please delete lines 24-33.

On page 5, line 34, please delete "3A and 3B", and insert --2A and 2B-- in place thereof.

On page 5, line 37, please delete "3A", and insert --2A-- in place thereof.

On page 6, line 13, please delete "3B", and insert --2B-- in place thereof.

On page 6, line 16, please delete "Figures 1 and 2", and insert --Figure 1-- in place thereof.

On page 6, line 23, please delete "Figure 4", and insert --Figure 3-- in place thereof.

On page 6, line 26, please delete "8-trimethyl" and insert --8-trimethylpsoralen-- in place thereof.

On page 6, line 27, after "5 AGGAAGGGGG3)", please insert --(SEQ ID NO:1)--.

On page 7, line 9, please delete "Figures 5A and 5B show", and insert --Figure 4 shows-- in place thereof.

On page 7, line 10, please delete "Figure 5A", and insert --Figure 4-- in place thereof.

On page 7, please delete lines 24-37.

On page 8, please delete lines 1-29.

On page 8, line 30, please delete "Figures 7A and 7B", and insert --Figures 5A and 5B-- in place thereof.

On page 8, line 34, please delete "7A", and insert --5A-- in place thereof.

On page 9, line 11, please delete "7B", and insert --5B-- in place thereof.

On page 9, please delete lines 18-37.

On page 10, line 1, please delete "Figure 9", and insert --Figure 6-- in place thereof.

On page 15, lines 10-11, please delete "as shown in Fig. 2".

On page 15, line 36, please delete "and is reproduced in Figure 2".

On page 15, line 36, please insert --The electrophoretic gel showed binding of the triplex forming oligonucleotide "AG10" to the supF gene target. To assay for triplex formation, ^{32}P-labeled oligonucleotides, either AG10 (^{5} AGGAAGGGGG^{3}) (SEQ ID NO:2) or the reverse sequence oligomer (GA10), were incubated with a 240 bp double-stranded fragment containing the entire supF gene. The products of the binding reactions were visualized by polyacrylamide gel electrophoresis and autoradiography.--

On page 16, line 1, please delete "As shown in Figure 2, binding", and insert --Binding-- in place thereof.

On page 16, line 2, please delete "(lane 2)".

On page 16, line 6, please delete "(lane 1)".

On page 16, line 7, please delete "(lane 3)".

On page 16, line 9, after "GGGGGAAGGA 3)", please insert --(SEQ ID NO:3)--, and delete "(lane 4)".

On page 16, line 11, please delete "(lane 5)".

On page 16, line 12, please delete "(lane 6)" and "(lane 7)".

On page 16, line 13, please delete "(lane 8)".

On page 17, lines 5-6, please delete "As shown in Figure 2,".

On page 18, line 9, after "(5 CCCCCTTC 3)", please insert --(SEQ ID NO:4)--.

On page 19, line 7, please insert --(SEQ ID NO:1)-- under "pso-^{5} AGGAAGGGGG^{3} ".

On page 19, line 8, please insert --(SEQ ID NO:5)-- under "pso-^{5} GGGGGAAGGA^{3} ".

On page 19, lines 9 and 10, please insert --(SEQ ID NO:1 and SEQ ID NO:4)-- under "pso-^{5} AGGAAGGGGG^{3} " and under "^{3} CTTCCCCC^{5} ", respectively.

On page 19, line 12, please insert --(SEQ ID NO:1)-- under "pso-^{5} AGGAAGGGGG^{3} ".

On page 20, line 8, please delete "Fig. 3a", and insert --Figure 2a-- in place thereof.

On page 20, line 35, please delete "Figure 3b", and insert --Figure 2b-- in place thereof.

On page 21, line 3, please delete "Fig. 3b", and insert --Figure 2b-- in place thereof.

On page 24, line 13, please delete "Fig. 4", and insert --Figure 3-- in place thereof.

On page 24, line 15, after "(5 AGGAAGGGGG3)", please insert --(SEQ ID NO:2)--.

On page 24, line 16, after "(5 GGGGGAAGGA3)", please insert --(SEQ ID NO:3)--.

On page 25, line 24, after "3)", please insert --(SEQ ID NO:6)--.

On page 25, line 25, after "TCC CCC 3)", please insert --(SEQ ID NO:7).

On page 27, line 21, please delete "Fig. 4", and insert --Figure 3-- in place thereof.

On page 27, line 28, after "5 AGGAAGGGGG3 ", please insert --(SEQ ID NO:2).

On page 28, lines 25-26, please delete "Fig. 5a and illustrated in Fig. 5b", and insert --Figure 4-- in place thereof.

On page 28, line 26, before "Digestion", please insert --A gel demonstrated site-specific formation of triplex DNA in the supF gene by psoralen-AG10 using a restriction enzyme protection assay. Analysis by agarose gel electrophoresis of *Hinf* I digestions of the 250 bp supF

gene PCR fragment under various conditions was done. The supF fragment was incubated with or without psoralen-AG10 at a 100-fold molar excess, treated by 1.8 J/cm^2 of UVA irradiation, and then subjected to *Hin*f I digestion.--.

On page 28, line 27, please delete "yields", and insert --yielded--.

On page 28, line 28, plese delete "(lane 1)".

On page 28, line 29, please delete "(lane 6)".

On page 28, line 31, please delete "(lane 3) results", and insert --resulted--.

On page 28, lines 33-34, please delete "demonstrated by the appearance of".

On page 28, line 34, after "fragment", please insert --appeared--.

On page 29, line 1, please delete "(lane 4)".

On page 29, line 3, please delete "(lane 2)".

On page 29, line 18, please delete "Fig. 6 illustrates", and insert --A gel experiment showed site-specific formation of triplex DNA in the SV40 vector as a function of the ratio of oligonucleotide to SV40 DNA. Binding of psoralen-AG10 as a triple strand to bp 167-176 of the supF gene within the SV40 vector was assayed by examining protection from *Hin*f I digestion at bp 164-168, as diagrammed in Figure 4. The SV40 vector containing the supF target gene (50nM) was incubated with psoralen-AG10 at ratios of oligomer to vector of from 1:1 to 1000:1, irradiated with 1.8 J/cm^2 of UVA, digested with *Hin*f I, and run on a 4.5% Nusieve gel. Because the sequences flanking the supF gene in the SV40 DNA differ from those in the PCR fragment,

and since there are multiple *Hinf* I sites in SV40, the pattern of bands is more complex.--

On page 29, line 19, please delete "that".

On page 29, line 23, please delete "(arrow)".

On page 29, line 33, please delete "Fig. 4", and insert --Figure 3-- in place thereof.

On page 31, line 12, please delete "Fig. 7A", and insert --Figure 5A-- in place thereof.

On page 31, line 16, please delete "Fig. 7", and insert --Figures 5A and 5B-- in place thereof.

On page 31, line 34, please delete "Fig. 7B", and insert --Figure 5B-- in place thereof.

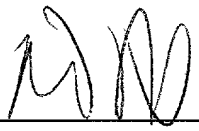
On page 33, line 11, after "sequences.", please insert --An analysis was done of supF gene mutations in the SV40 vector by a colony hybridization assay. Bacterial colonies containing SV40 plasmid vector DNA carrying supF gene mutations were grown and lysed *in situ* on nylon filters to allow nucleic acid hybridization. Oligonucleotide probes that either exactly matched the wild type sequence of the supF gene at base pairs 158-176 or matched the sequence of the 167 T:A to A:T transversion mutation at those base pairs were radioactively labeled and allowed to hybridize with duplicate filters under conditions designed to enable discrimination between mutant and wild type sequences. Binding was visualized by autoradiography.--.

On page 33, lines 11-13, please delete "The results of one such analysis are shown in Fig. 8. Of the 19 colonies assayed in this particular experiment," and insert --Results showed in this particular experiment that of the 19 colonies assayed,-- in place thereof.

On page 33, line 16, please delete "in the upper right hand corner".

On page 36, line 8, please delete "Figure 9", and insert --Figure 6-- in place thereof.

Respectfully submitted,



Robert A. Hodges
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Date: February 14, 2001

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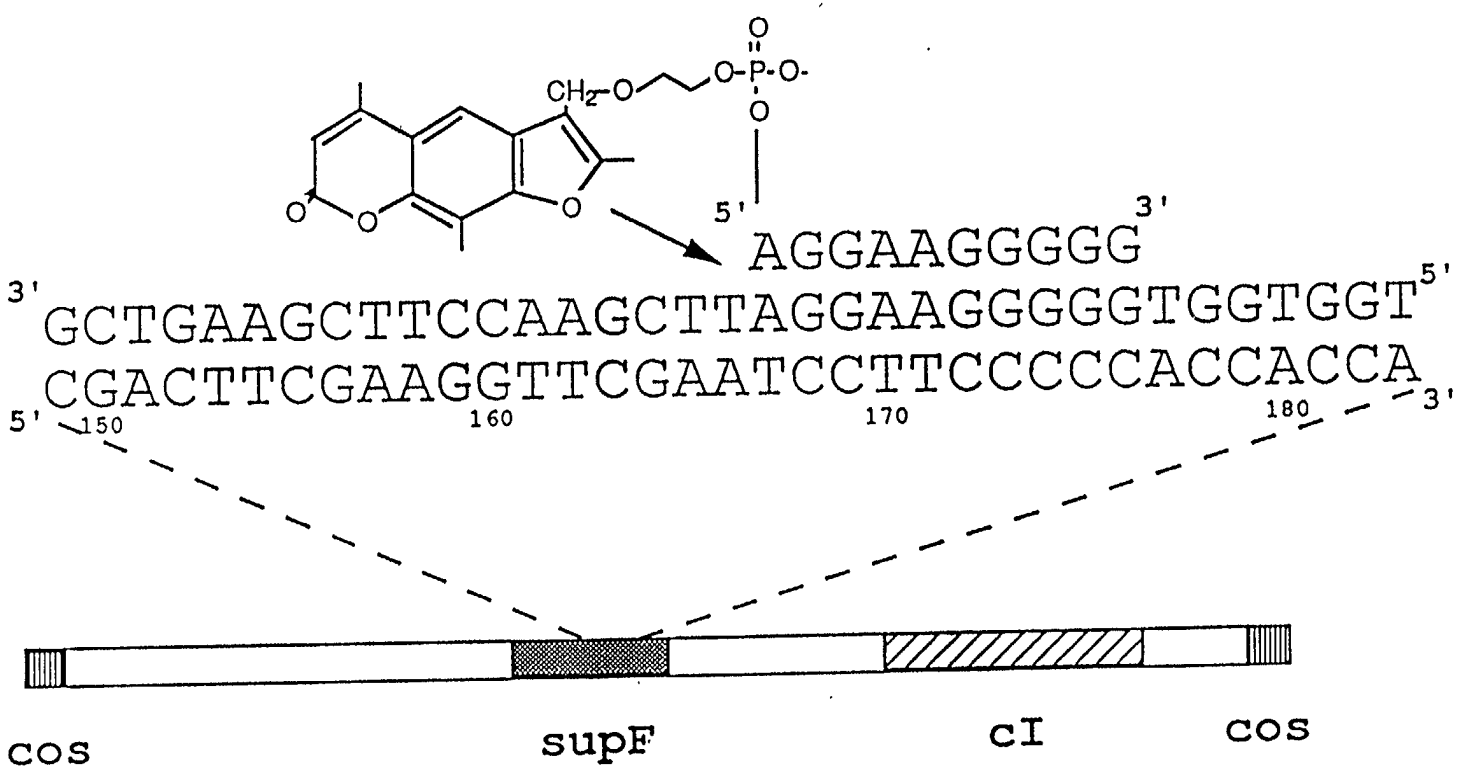

CH2-O
O-P-O-
O
5'
3'
AGGAAGGGGG
3'
GCTGAAGCTTCCAAGCTTAGGAAGGGGGTGGTGGT
5'
CGACTTCGAAGGTTCGAATCCTTCCCCCACCACCA
3'
5'
150
160
170
180
cos
supF
cI
cos

Figure 1

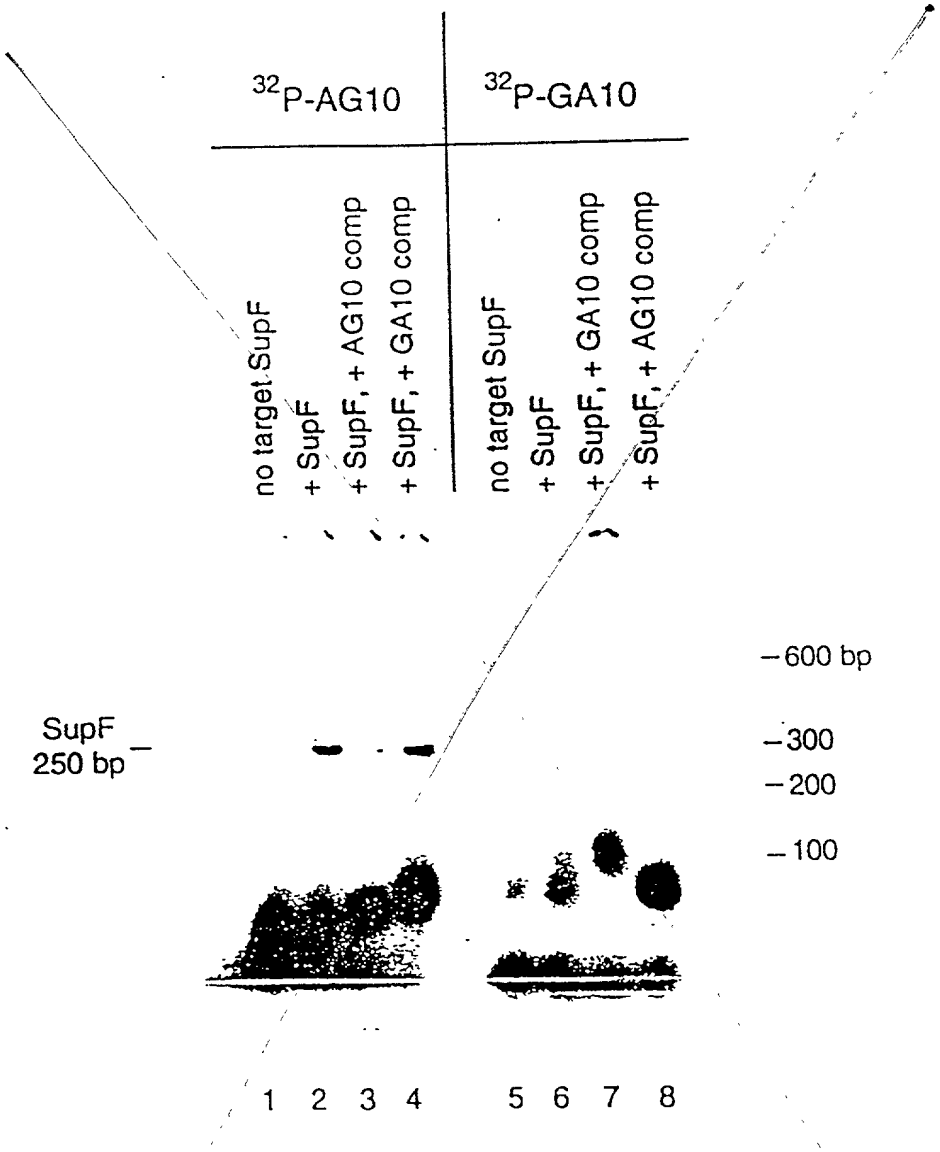

^{32}P-AG10
^{32}P-GA10
no target SupF
+ SupF
+ SupF, + AG10 comp
+ SupF, + GA10 comp
no target SupF
+ SupF
+ SupF, + GA10 comp
+ SupF, + AG10 comp
SupF
250 bp
600 bp
300
200
100
1 2 3 4 5 6 7 8

Figure 2

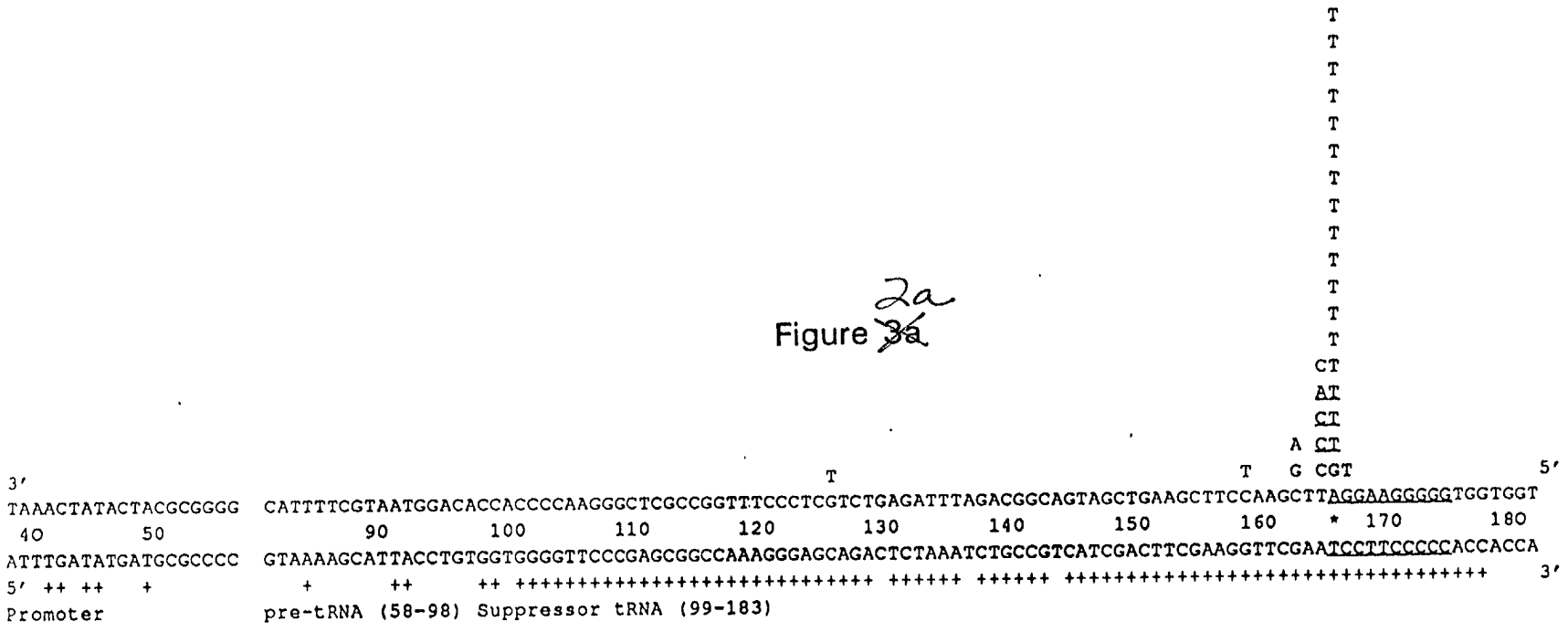


Figure 2a

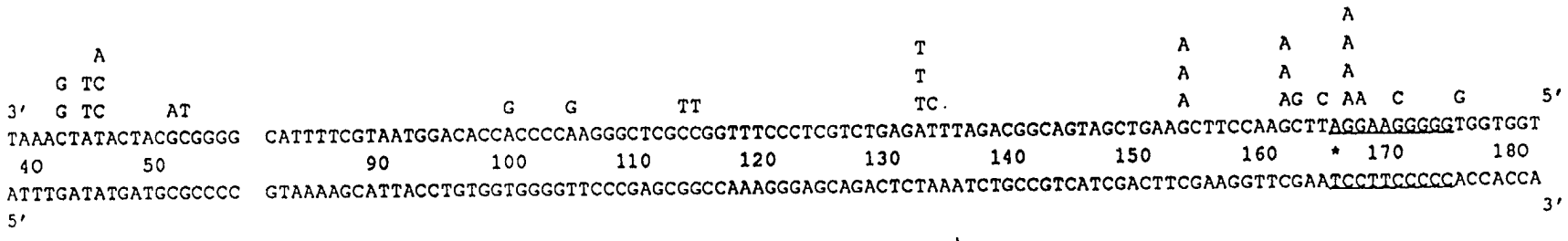


Figure 2b

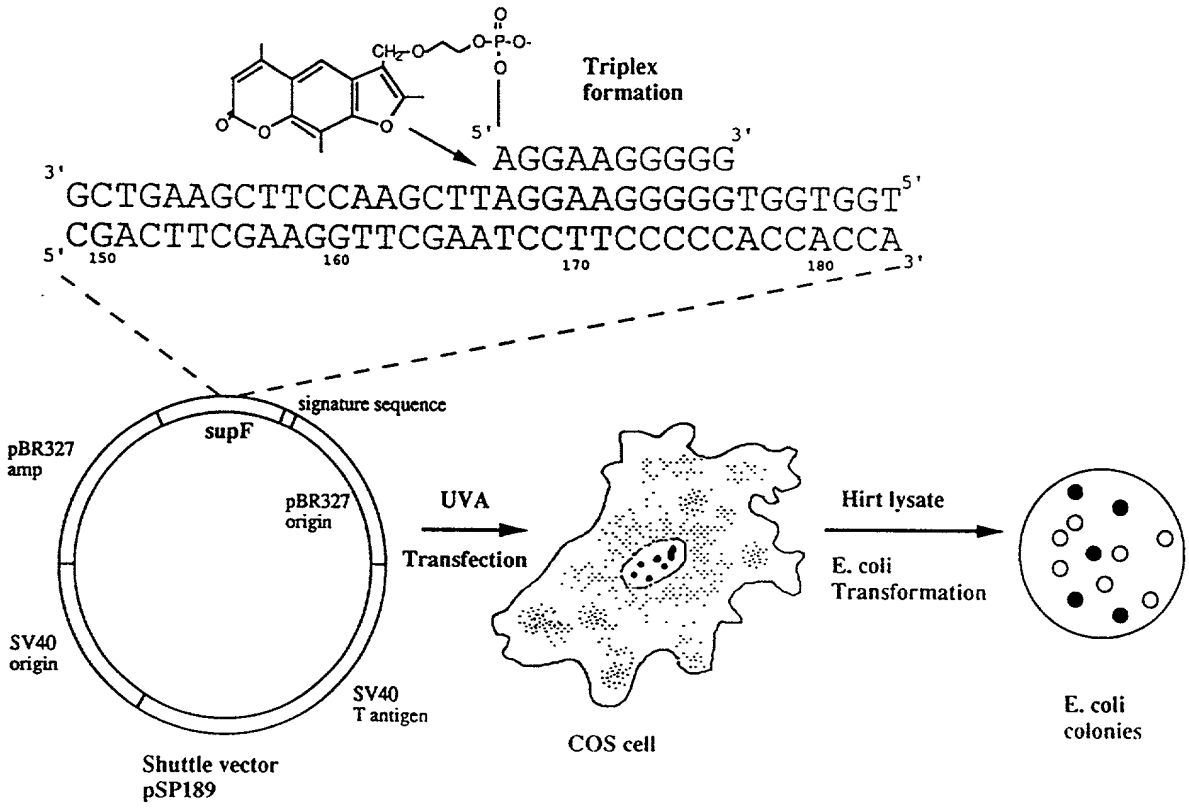
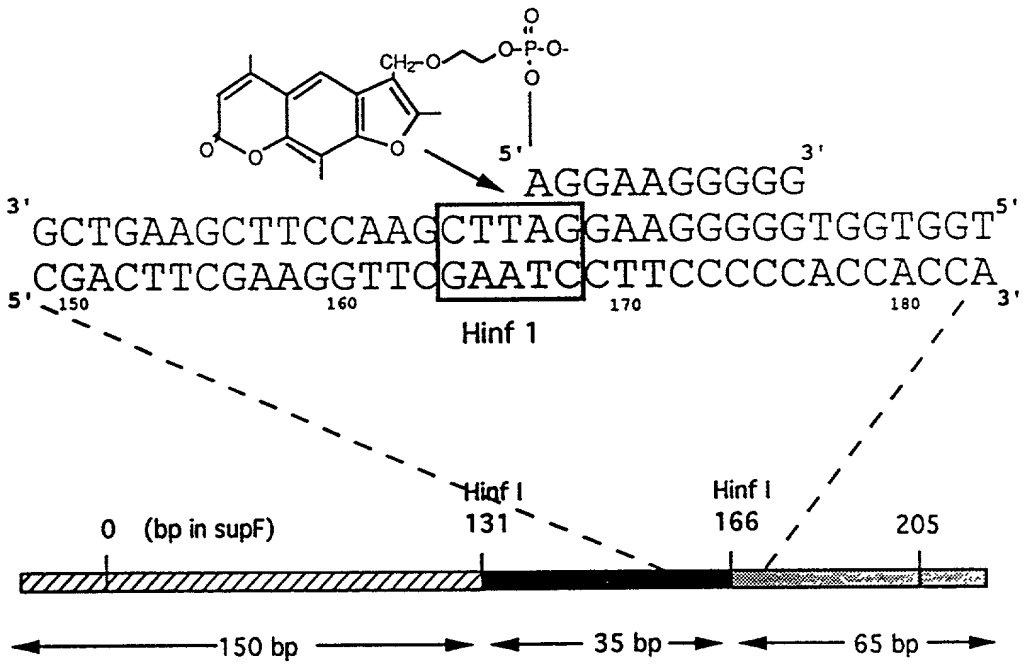

Triplex formation
AGGAAGGGGG
GCTGAAGCTTCCAAGCTTAGGAAGGGGGTGGTGGT
CGACTTCGAAGGTTCGAATCCTTCCCCCACCACCA
150
160
170
180
signature sequence
supF
pBR327 amp
pBR327 origin
SV40 origin
SV40 T antigen
Shuttle vector pSP189
UVA
Transfection
COS cell
Hirt lysate
E. coli Transformation
E. coli colonies

Figure ~~4~~ 3



supF PCR fragment: 250 bp

complete Hinf I digestion: 150 bp, 65 bp, and 35 bp
Hinf I site at 164-168 blocked or mutated: 150 bp and 100 bp

Figure ~~5a~~ 4

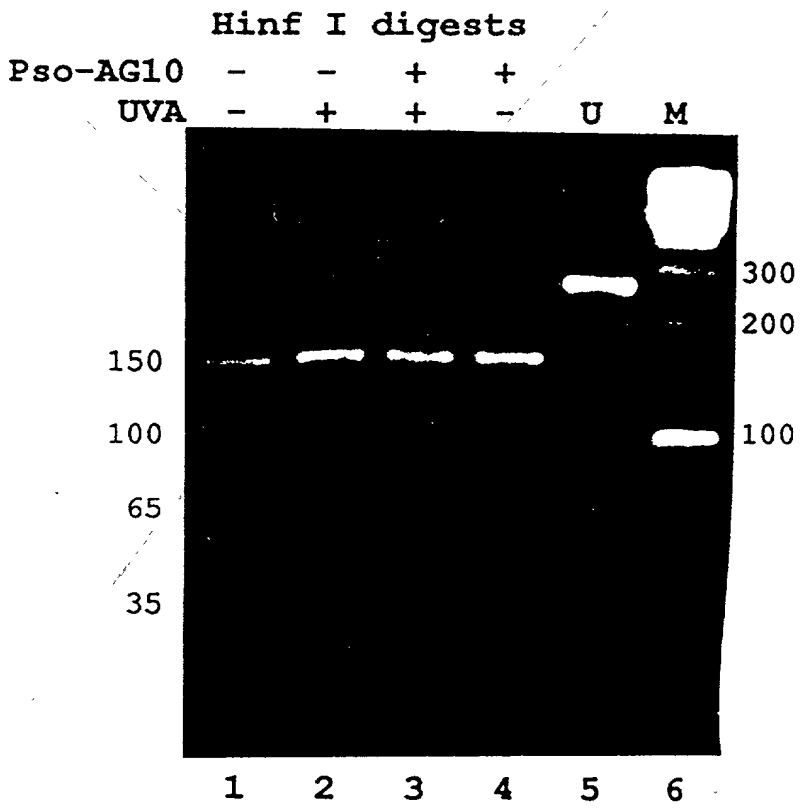


Figure 5b

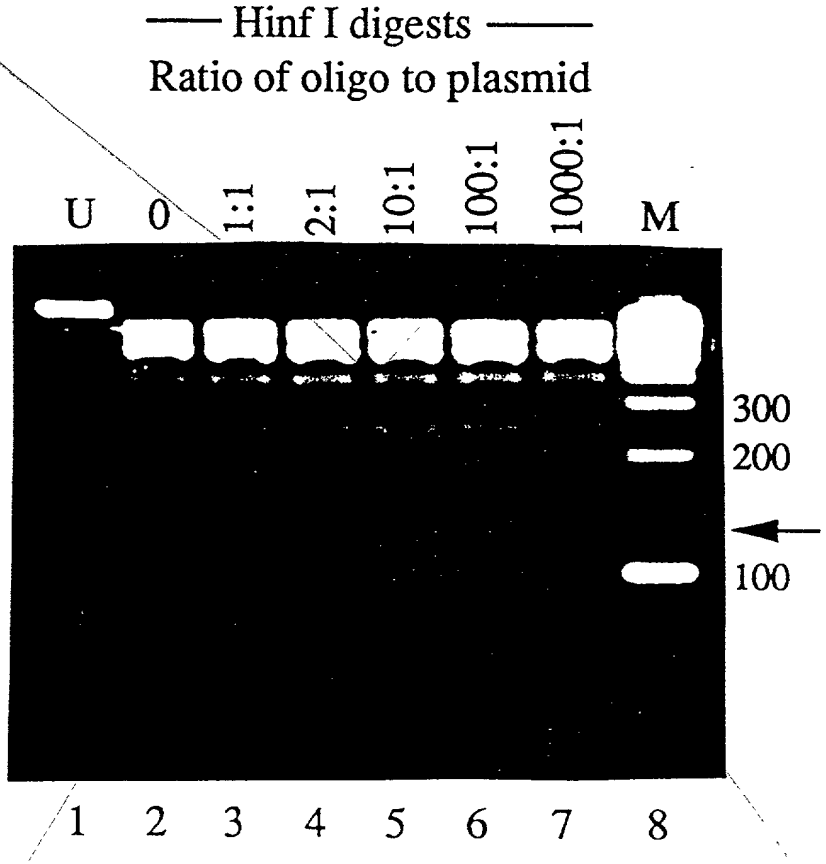

Hinf I digests
Ratio of oligo to plasmid
U
0
1:1
2:1
10:1
100:1
1000:1
M
300
200
100
1
2
3
4
5
6
7
8

Figure 6

Figure 5A

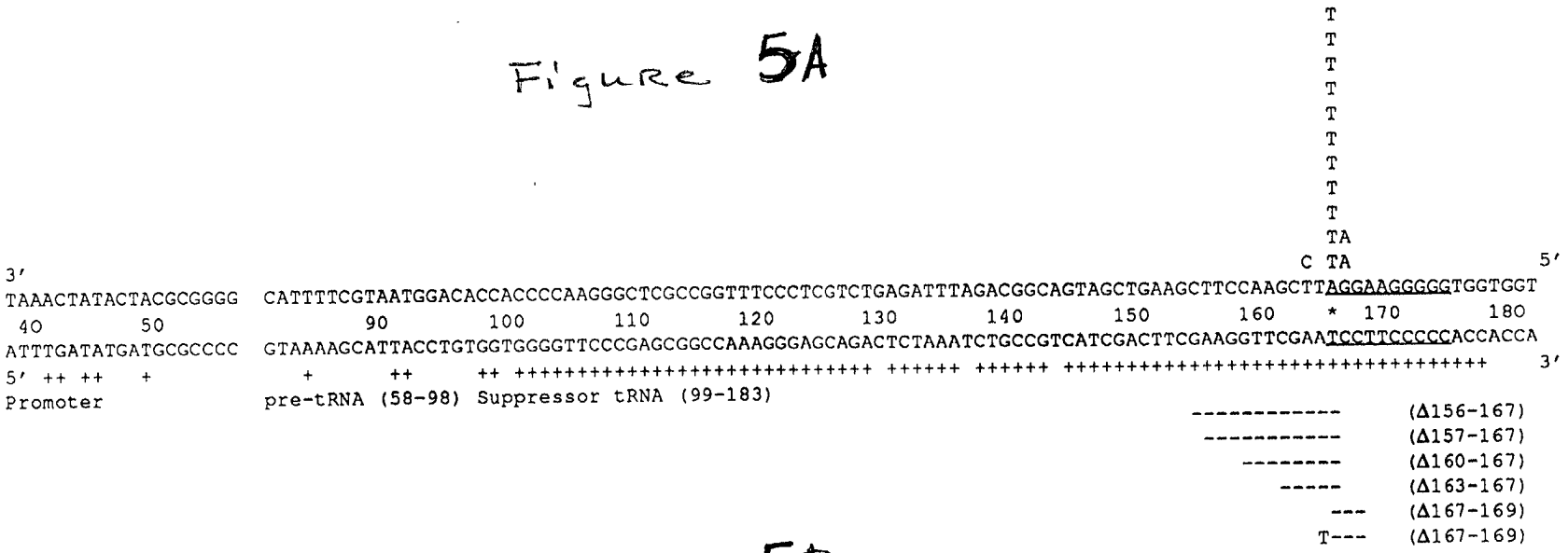


Figure 5B

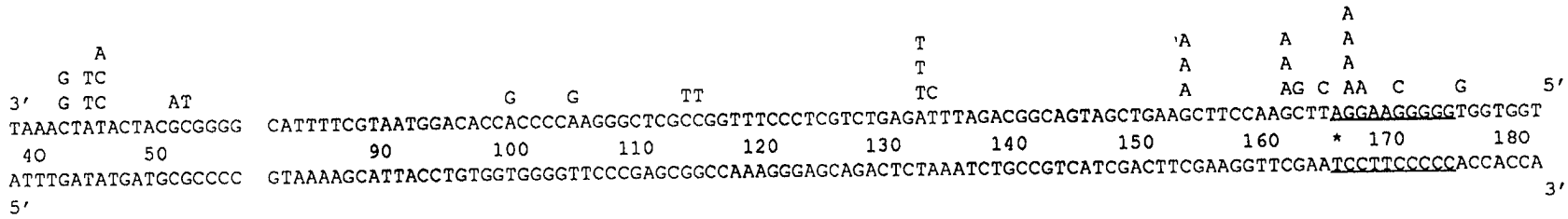


Figure 7

Colony hybridization assay

Wild type probe

Mutant probe bp 167 T->A

Figure 8

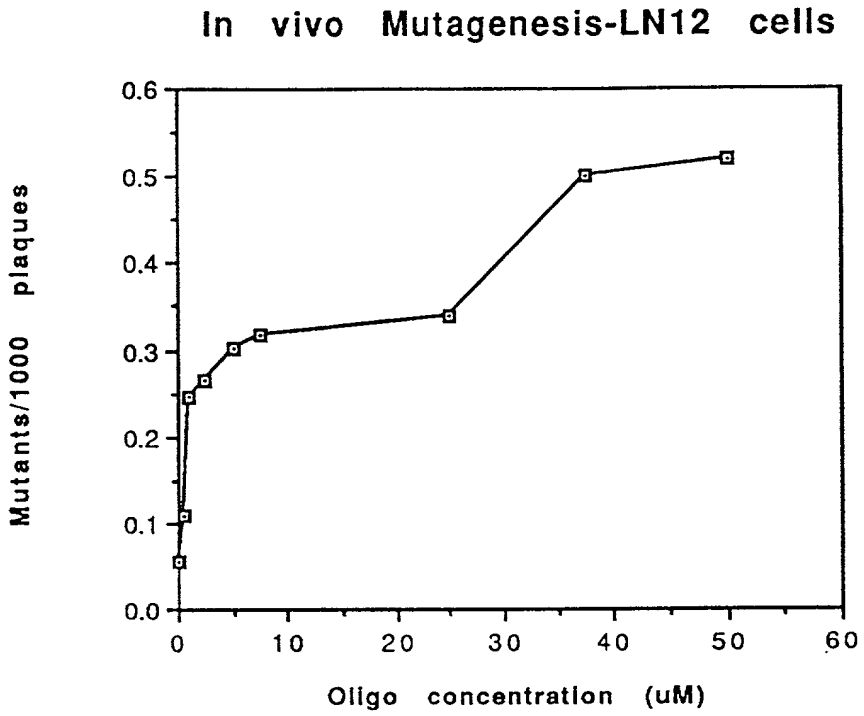


Figure 9

Figuee 6